1 **Measures of oxidative state are primarily driven by extrinsic factors in a long-distance**

2 **migrant**

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14

15 **Abstract**

16 Oxidative stress is a likely consequence of hard physical exertion, and thus a potential

17 mediator of life-history tradeoffs in migratory animals. However, little is known about the

18 relative importance of intrinsic and extrinsic stressors on the oxidative state of individuals in

19 wild populations. We quantified the relationships between air temperature, sex, body

20 condition, and three markers of oxidative state (malondialdehyde, superoxide dismutase

21 and total antioxidant capacity), across hundreds of individuals of a long-distance migrant

22 (the brent goose *Branta bernicla hrota*) during wintering and spring staging. We found that

23 air temperature and migratory stage were the strongest predictors of oxidative state. This

24 emphasizes the importance of extrinsic factors in regulating the oxidative state of migrating

25 birds, with differential effects across the migration. The significance of abiotic effects

26 demonstrates an additional mechanism by which changing climates may affect migratory

27 costs.

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29 **Key Words**

30 Antioxidants; Avian; Body Mass; Carry-over Effect; Reproduction

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32 **Introduction**

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34 A migratory life style places huge physiological demands on individuals [1]. While there is

35 some understanding of the costs of such behaviour, large gaps remain. One such case is

36 oxidative stress, a measure of the oxidative damage caused to important biomolecules,

37 which is an important physiological stressor [2–4]. Metabolic processes naturally result in

38 the formation of reactive oxygen species (ROS) and conditions that increase metabolic rate

39 have the potential to increase oxidative damage [2]. This means that upregulation of

40 antioxidant defences or repair mechanisms could occur to counter the oxidative cost of

41 migration, yet antioxidants are also required for reproductive investment [2,5]. Therefore,

42 the antioxidant system should play a key role in mitigating some of the costs of migration

43 [2,6,7], with differences in oxidative state affecting variation in survival and reproductive

44 performance, [2,3,5,8,9].

45

46 Although studies of captive animals have highlighted the potential impacts of oxidative

47 stress [2–4], such benign environments may not reveal the true costs faced by wild

48 organisms, where a range of factors interact to affect their oxidative state [2,4,10].

49 Migratory animals face significant oxidative burdens resulting from annual movements, and

50   must also face locations that differ in both biotic and abiotic conditions. Yet there is limited

51   information on the impacts of migration or tradeoffs between internal and external states,

52   although studies suggest active responses occur within the redox system [6,7].

53

54   Here we examine the relative importance of intrinsic (biotic) and extrinsic (abiotic and

55   biotic) factors in affecting the oxidative state of light-bellied brent geese *Branta bernicla*

56   *hrota* (LBBG) using data collected across three consecutive years on >350 individuals on the

57   wintering and staging grounds. This species is a capital breeder that gains >50% of its

58   bodyweight to fuel extremely long migrations (> 12 000 km annually) and reproductive

59   attempts, fueled through the catabolism of fats that are highly sensitive to oxidative

60   damage [2,3,11]. In addition, climatic conditions significantly affect the survival and

61   breeding success of individuals [11,12], making this an excellent system to quantify the

62   relationship between individual body condition, weather and three frequently used markers

63   of oxidative state.

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70 **Methods**

71 *Study System and Data Collection*

72 LBBG from the study flyway overwinter around coastal Ireland, migrating to Arctic Canada

73 via staging grounds in Iceland. We captured birds using cannon nets around Dublin Bay,

74 Ireland (53.33N, 6.13W) from February – April, and from Iceland (Alftanes peninsula,

75 64.10N, 22.01W) in May, in 3 consecutive years. LBBG were held individually in sacks then

76 ringed, had standard morphometrics taken (see electronic supplementary material [ESM])

77 and blood drawn from the tarsal vein. This sample was separated into plasma and red blood

78 cells by centrifugation (12000 g for 3 mins), flash frozen in liquid nitrogen within 6 min of

79 collection, stored at -80 °C, and analysed within 6 months.

80

81 *Explanatory Variables: Biotic and Abiotic Factors*

82 *Scaled Body Mass*

83 Mass in migratory birds is highly variable and strongly non-linear. As such we followed the

84 methods of Harrison *et al* [11] to calculate a seasonally corrected estimate of mass that is

85 independent of individual body size (see ESM), referred to hereafter as 'scaled mass'.

86

87 *Sexing of Individuals*

88 A discriminant function based on morphological differences was used to classify birds as

89 male, female or undetermined (if in a small overlapping zone). All 'undetermined' birds

90 were molecularly sexed by a commercial company (Animal Genetics, St Austell, UK).

91

92 *Weather*

93   Mean daily temperature for Feb – Apr in all study years were obtained from MET Éireann,

94   with similar data for May obtained from Icelandic Met Office stations. We examined a range

95   of temperature metrics but present here results using a two day average of mean

96   temperature (hereafter 'temperature') as the different metrics did not produce qualitatively

97   differing results (see ESM Tables S1, S2).

98

99   *Response Variables: Measures of Oxidative State*

100   *Oxidative Damage to Lipids*

101   Plasma concentrations of malondialdehyde (MDA) were determined using high performance

102   liquid chromatography following Nussey *et al* [7] (see ESM), and are expressed in μM. A

103   subset of samples run in duplicate showed high repeatability (R = 0.91 (95% CRI 0.85 - 0.94)

104   n = 136).

105

106   *Superoxide Dismutase Activity*

107   Total SOD activity in plasma was determined using a colorimetric assay kit (Cayman

108   Chemicals, Michigan, USA). Repeatability of the assay was high (R = 0.82 (95% CRI 0.77 -

109   0.85), n = 584), and results are expressed in units/ml.

110

111   *Residual Total Antioxidant Capacity*

112   Total non-enzymatic antioxidant capacity of plasma samples was measured using a Cayman

113   Chemicals colorimetric assay kit and spectrophotometer, with high repeatability (R = 0.81

114   (95% CRI 0.75 - 0.85), n = 798). Results are expressed as Trolox-equivalent antioxidant

115   concentrations (TEAC, mM). As TEAC levels have been reported to be strongly dependent on

116   uric acid concentration [13], plasma concentrations of uric acid were determined using a

117 third Cayman Chemicals assay kit and spectrophotometer. Assay repeatability was good,

118 and comparable with other studies (R = 0.73 (95% CRI 0.66 - 0.79), n = 234). Residuals from

119 a linear model using uric acid as the predictor and TEAC as the response variable were used

120 as a measure of plasma antioxidants excluding the effects of uric acid [13], hereafter

121 'RTEAC'.

122

123

124 *Statistical Analysis*

125 To test for the combined effects of biotic and abiotic factors on LBBG oxidative stress

126 measures, we fitted separate general linear mixed models with each oxidative state

127 measure in turn as the response variable. The full model included all three-way interactions

128 between the fixed effects of migration stage (the country of capture), scaled mass, sex and

129 temperature, and the random effect of year. Model simplification was conducted using an

130 information theoretic approach. Goodness of fit estimates were calculated using a

131 likelihood-ratio based pseudo-R-squared [14].

132

133 **Results**

134

135 Samples were collected from 356 adults across three years (2014, n = 104; 2015, n = 181;

136 2016, n = 71) on the wintering (n = 195) and staging (n = 161) grounds. Candidate model sets

137 provided good explanatory power for MDA and SOD but not RTEAC (Table 1, Table S3). For

138 MDA the clear top model revealed opposing relationships between migratory stages in

139 relation to temperature, with warmer temperatures resulting in elevated damage levels on

140 wintering grounds, but with the opposite relationship on the staging grounds (Table 1, Fig

141 1). While similar factors featured additively in competing candidate models for antioxidant

142 metrics, the null model was the most appropriate in both cases. There was negligible

143 support for intrinsic factors as explanatory variables for any oxidative state measures.

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Oxidative Status Measure	Log likelihood	AICc	ΔAICc	ω	Random Effect Variance Year / Residual	Adj R^2
MDA						
Migration stage * Temperature	-312.00	636.2	0.00	0.87	0.378 / 0.314	0.533
Migration stage * Temperature + Sex	-312.98	640.3	4.04	0.12	0.376 / 0.313	0.536
Migration stage * Temperature + Migration stage * Sex	-313.86	644.1	7.89	0.02	0.376 / 0.313	0.536
SOD						
Null	-356.81	719.7	0.00	0.60	1.675 / 0.416	0.670
Temperature	-356.67	721.4	1.76	0.25	1.671 / 0.409	0.677
Migration stage + Temperature	-356.50	723.2	3.49	0.10	1.642 / 0.406	0.681
RTEAC						
Null	-342.27	690.6	0.00	0.63	0.006 / 0.394	0.002

Migration stage	-341.94	692.0	1.37	0.32	0.003 / 0.391	0.017
Mass + Sex	-342.61	695.4	4.78	0.06	0.0003 / 0.378	0.065

Table 1. Top candidate models explaining variation in oxidative damage (MDA) and

antioxidants (SOD, RTEAC) in LBBG. ΔAICc = difference compared to top model, ω = model
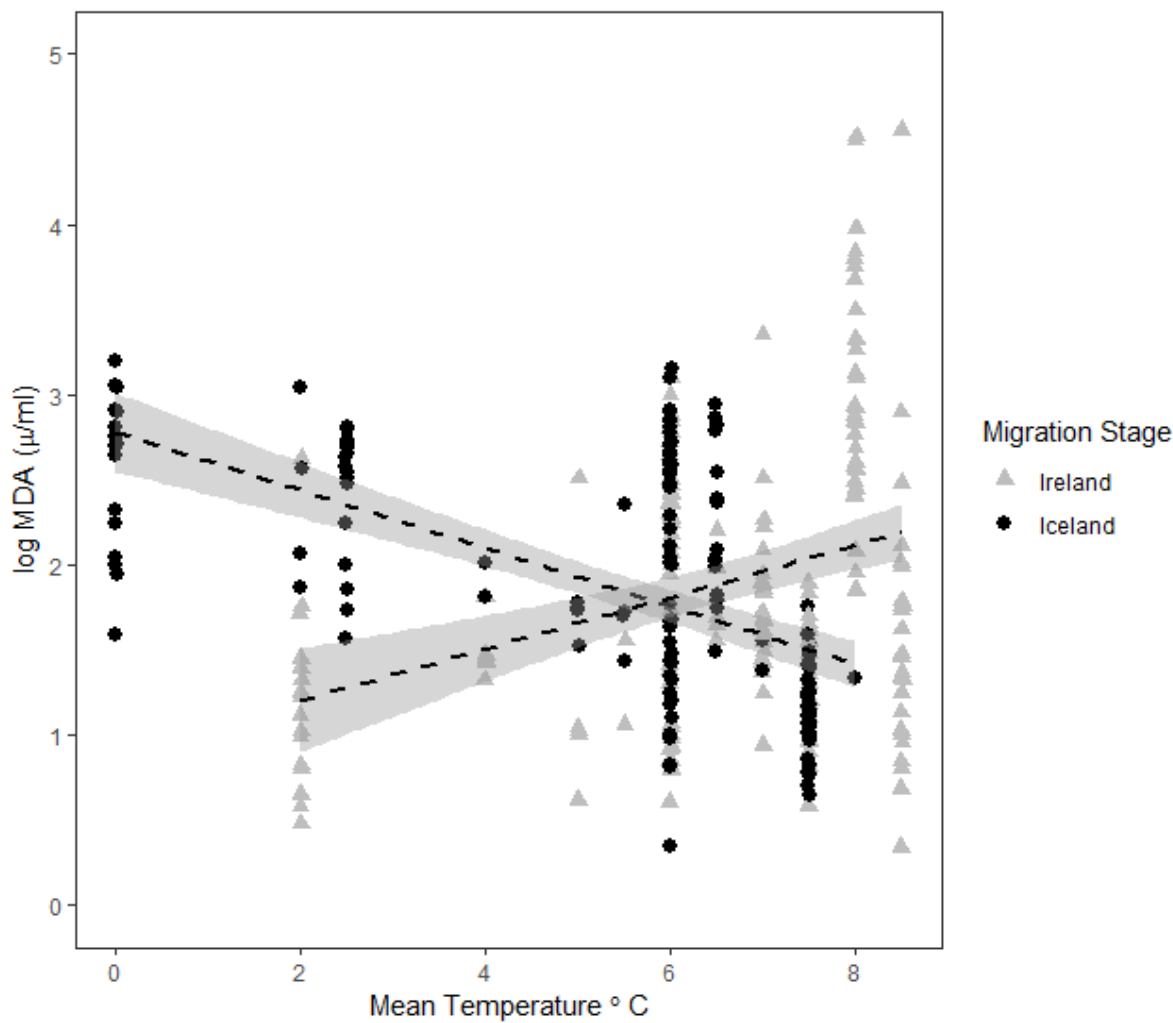
weighting, Adj R^2 = model fit.

Figure 1. Lipid damage (MDA) varies significantly with temperature between migration

stages. Lines represent linear regressions with 95% confidence intervals.

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Discussion

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162   We examined, for the first time, the oxidative burdens faced by a capital breeder during

163   migration, and found little effect of intrinsic factors. This is surprising since their life history

164   should make LBBG highly susceptible to oxidative stress. Instead, air temperature and

165   migration stage were the strongest influences on measures of oxidative damage,

166   highlighting the role abiotic and extrinsic biotic factors play in regulating the oxidative state

167   of migrating birds.

168

169   Weather conditions are known to substantially affect migratory behaviour in many species

170   [1,11], and we found a positive relationship between temperature and oxidative damage on

171   the wintering grounds. As an Arctic breeder, selection is likely to favour an ability to cope

172   with cold spells rather than warm ones, so increasing temperatures as birds prepare to

173   migrate potentially further raises metabolic rate, with consequent increases in lipid damage.

174   Exposure to increased temperatures has been shown to increase MDA levels in domestic

175   chickens *Gallus gallus* [15]. However, in contrast, the reverse relationship was seen on the

176   staging grounds, where this may be an indirect result of variation in resource availability

177   across stages. On the wintering grounds there is widespread availability and exploitation of

178   maintained grasslands [16]. However, during migration, geese typically overtake the green

179   wave of vegetation growth, so cooler temperatures on the staging grounds will frequently

180   reduce available food via reduced/no plant growth or increased snow cover. Such

181   reductions in food availability have been shown to lead to increases in oxidative damage

182   and decreases in plasma antioxidants in tree swallows *Tachycineta bicolor* [17].

183

184   In contrast to the significant effects seen with for oxidative damage, a null model was the

185   top model for both measures of antioxidant levels. As obligate high-throughput grazers,

186   LBBG have little ability to switch diets, even to seeds or fruits, to replenish antioxidant

187   stores, so increasing circulating antioxidant levels would require upregulation of

188   endogenous reserves, with associated metabolic costs [2]. LBBG may thus tolerate a

189   transient oxidative burden, reserving antioxidants for the longer migration leg and

190   reproductive attempt to come.

191

192   The absence of any significant effects of sex or mass on LBBG's oxidative state may reflect

193   adaptations to an extreme migration that has limited scope for temporal buffering. LBBG

194   breeding success is highly stochastic, so tradeoffs are likely to shift towards adult survival

195   over reproductive investment in this long-lived species [11,12]. However, sampling of

196   individuals on their breeding grounds, or consideration of unmeasured markers, may have

197   revealed allocation differences between the sexes. Although a higher scaled mass is a crucial

198   predictor of reproductive success [11], we found no evidence that LBBG increased

199   circulating levels of antioxidants to protect lipid reserves. The absence of relationships

200   suggests that LBBG, and potentially other long-distance migrants, may be adapted to

201   managing elevated pro-oxidant levels. While long-distance flights can increase oxidative

202   damage [2,7], waterfowl are obligate powered fliers, so are likely to be adapted to this

203   stressor. LBBG also have a short breeding window that necessarily selects for rapid chick

204   growth, a process linked to increased levels of ROS [18]. This early life history may prime

205   LBBG to successfully manage ROS burdens throughout their lifespan [3,10].

206

207 These results highlight a further mechanism by which a changing climate may affect Arctic

208 migrants, with increasing temperatures affecting their physiology. Extrinsic factors strongly

209 influence aspects of the annual cycle of migratory species [1,12,19], often through carry-

210 over effects [19]. Changing temperatures may thus create physiological carry-over effects

211 [5,10], a mechanism that warrants further investigation, particularly in relation to how

212 individuals manage oxidative damage during migration.

213

214 **Ethics**

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216 All birds were captured and handled under country-specific licenses: Ireland (NPWS

217 0282016, NPWS 0322014), UK (HO Licence: PPL30/3205), Iceland (IINH 414).

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219 **Data accessibility**

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221 Data are available from the Dryad Digital Repository

222 https://doi.org/10.5061/dryad.j4k3t6f/1 [20].

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224 **Authors' contributions**

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226 SB and JB conceived the study. TWB, IRC, FV, KM & SB collected data, with analyses

227 performed by TWB and IRC. TWB, SB & JB drafted the initial manuscript, and all authors

228 contributed to subsequent revisions, approved the final version and agreed to be

229 accountable for all aspects of the work.

230

246 **Footnotes**

247 Electronic supplementary material is available online at …….

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